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1. Conner et al. (1983) PNAS 80: 278-282.
2. Rollini et al. PNAS (1985 Nov) 82(12): 7197-7201.
3. Gorski et al. IMMUNOGENTICS (1987) 25(6):379-402.
4. de Preval et al. IMMUNOGEENTICS (1987) 26(4-5): 249-257.
5. Irle et al. J. EXPERIMENTAL MEDICINE (1988 Mar 1) 167(3): 853-872.
6. Andersson et al. IMMUNOGENETICS (1988) 28 (1): 1-5.

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# The single *DR<sub>β</sub>* gene of the *DRw8* haplotype is closely related to the *DR<sub>β</sub>III* gene encoding DRw52

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**Abstract.** In most individuals two *HLA-DR<sub>β</sub>* genes are expressed from each chromosome. One of these genes encodes one of the classical DR specificities, while the other encodes either of the supertypic DRw52/DRw53 specificities. In addition to these genes usually one or two *DR<sub>β</sub>* pseudogenes are present. In contrast, the *DRw8* chromosomal region only contains a single *DR<sub>β</sub>* gene. To determine the relationship of this single gene to the multiple *DR<sub>β</sub>* genes of other DR specificities, comparisons of Southern genomic blots were carried out. In this analysis genomic clones for each individual *DR<sub>β</sub>* chain locus were included. The *DR<sub>β</sub>w8* gene was indistinguishable from the *DR<sub>β</sub>III* gene of DR3 cells (encoding DRw52), suggesting that it is closely related to the latter gene. The functional implications of this finding are discussed.

## Introduction

Class II histocompatibility antigens in man are encoded by the *HLA-D* region. Serological, cellular, and, more recently, molecular cloning analyses have shown that this region is composed of three major subregions, *HLA-DR*, *-DQ*, and *-DP*. From each subregion at least one class II molecule consisting of an  $\alpha$  and a  $\beta$  chain is expressed (Albert et al. 1985). The class II antigens are extremely polymorphic (Bach 1985). A large number of serologically defined DR specificities are known. By the use of cellular methods several of these specificities have been further split. These specificities correspond to allelic series of *DR<sub>β</sub>* chains, while the *DR<sub>α</sub>* chain is invariable. Two separate allelic series of DR specificities, the classical DR and DRw52/DRw53, have been established by serological

studies. The DR series involves a large number of alleles, while the DRw52/DRw53 polymorphism is much more restricted (reviewed in Mach et al. 1986). The DRw52 specificity is carried by DR3, 5, w6, and w8 individuals, whereas those with DR specificities 4, 7, and w9 have the DRw53 specificity. More than one *DR<sub>β</sub>* chain is expressed from each chromosome for most of the DR specificities (Rollini et al. 1985, Giles and Capra 1985). Epitopes corresponding to the supertypic specificities DRw52 and DRw53 are present on  $\beta$  chains separate from those displaying the classical DR specificities (Tanigaki and Tosi 1982).

In addition to the sequence variability between *DR* alleles there is a variability in the number of *DR<sub>β</sub>* genes (Böhme et al. 1985). Molecular cloning and genomic hybridizations have shown that three *DR<sub>β</sub>* genes, designated I, II, and III, are present in the DR3, 5, and w6 haplotypes (Rollini et al. 1985). *DR<sub>β</sub>I* encodes the DR3 specificity, *DR<sub>β</sub>III* corresponds to the DRw52 epitope (Gorski et al. 1985), while *DR<sub>β</sub>II* is a pseudogene. In the DR4 haplotype four *DR<sub>β</sub>* genes exist, two of which are pseudogenes, while the other two encode the DR4 and DRw53 specificities. Hybridization experiments have shown that the DR7 and DRw9 haplotypes have a gene organization similar to DR4 (Böhme et al. 1985, Andersson et al. 1987). These studies also indicated that the DR2 haplotype contains three and the DR1 haplotype two *DR<sub>β</sub>* genes. In the DRw8 haplotype a single *DR<sub>β</sub>* gene has been found (Böhme et al. 1985). The present study was undertaken to clarify the relationship of this single gene to the multiple genes encountered in the DR3 and DR4 haplotypes. Since detailed molecular information is available from the cloned *DR<sub>β</sub>* genes of the DR haplotypes DR3 and DR4 it is possible to assign every single restriction fragment hybridizing with a *DR<sub>β</sub>* probe in Southern hybridizations of restricted DNA of these haplotypes to a specific gene. To minimize allelic restriction site polymorphism which might make such a comparison impossible, we decided to use hybridization probes corresponding

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to the reasonably wellconserved 3' portions of the  $DR_{\beta}$  genes.

## Materials and methods

**DNA sources.** DNA was prepared from the following homozygous typing cells that were selected from the typing panels used at the State Institute for Blood Group Serology, University Hospital, Linköping, Sweden: PH, DR1/1; IML, DR2/2; DL, DR3/3; SA, DR5/5; and IH, DR7/7.

Cell lines SPL, DRw8/w8 and KOZ, DRw9/DRw9 were obtained from Dr. J. Bodmer of the Tissue Antigen Laboratory, Imperial Cancer Research Fund Laboratories, London, England. Additional DNA was prepared from the cells used in our earlier work: BO, DR4/4 (Andersson et al. 1987) and GP, DRw6/w6 (Böhme et al. 1985).

**Hybridization probes.** Two  $DR_{\beta}$ -specific probes corresponding to (i) the cytoplasmic exon with flanking introns and (ii) the  $DR_{\beta}$  3'-untranslated exon were used (Table 1). After restriction enzyme digestions of the clones, the probe fragments were isolated by preparative agarose gel electrophoresis. Fragments were radioactively labeled to high specific activity with  $\alpha$ -( $^{32}$ P) deoxycytidine triphosphate by either nick-translation (Rigby et al. 1977) or random priming (Feinberg and Vogelstein 1984).

**DNA preparation and Southern blot analysis.** DNA was isolated from either 5 ml of peripheral blood according to the method of Böhme and

co-workers (1983) or 500  $\mu$ l of pelleted cultured cells as described by Grosfeld and co-workers (1982). Samples of 10  $\mu$ g of human DNA were digested to completion with restriction enzymes according to the manufacturer's recommendations. The digests were separated by agarose gel electrophoresis in 0.7 and 1.5% gels, in 1  $\times$  TBE (89 mM Tris-borate buffer, pH 8.3, 2.5 mM ethylenediaminetetraacetate). Southern transfer of restricted DNA to Biotrans hybridization membranes (Pall) was made overnight with 1  $\times$  SSC (0.015 M sodium citrate buffer, pH 7.0, 0.15 M NaCl). Hybridizations were carried out in 40% formamide at 42  $^{\circ}$ C. Washings were performed at high stringency (0.2  $\times$  SSC, 0.25% sodium dodecyl sulfate) at 58  $^{\circ}$ C for 1 h.

## Results and Discussion

To define the relationship of the  $DR_{\beta}$  gene carried by DRw8 individuals to those of the  $DR3$  and  $DR4$  haplotypes, genomic DNA samples from homozygous typing cells of DR specificities 1 to w9 were separately digested with Pst I, Bam HI, and Hind III and hybridized to a probe corresponding to exon 5 of  $DR_{\beta}$  genes (Table 1). Hybridization to Hind III-digested DNA is shown in Figure 1. In all cases a single hybridizing fragment was found for the DRw8 cells, which is in agreement with the proposal that this haplotype contains only a single  $DR_{\beta}$  gene. A single  $DR_{\beta}$  fragment in DRw8 cells was also obtained when genomic DNA digested with Pvu II was

Table 1. Documentation of hybridization probes

Probe corresponding to	Type of clone	Designation of clone	Restriction sites employed	Size of probe fragment (bp)	Reference
$DR_{\beta}$ exon 5 and intron	Genomic	p801-2	Bgl II	376	Larhammar et al. 1985
$DR_{\beta}$ exon 6	cDNA	pII- $\beta$ -4	Pst I	220	Gustafsson et al. 1984

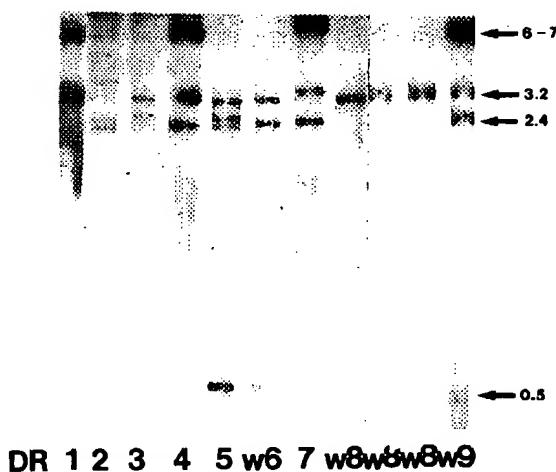


Fig. 1. Hybridization with the  $DR_{\beta}$  exon 5-specific probe to Hind III-restricted human genomic DNA corresponding to DR specificities 1 to w9. The sizes of restriction fragments are given in kilobases

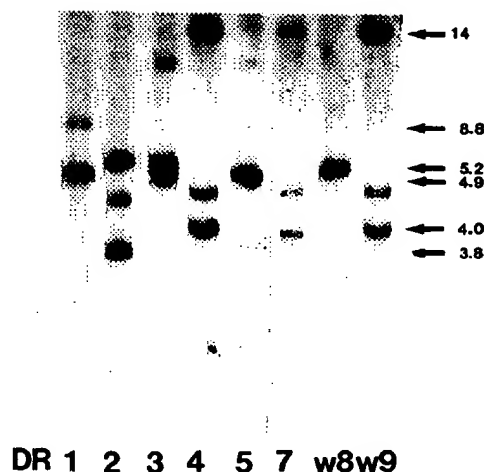


Fig. 2. Restriction fragment patterns of human genomic DNA, representing the same individuals as in Figure 1, digested with Pvu II and hybridized with the  $DR_{\beta}$  3'-untranslated probe. The sizes of restriction fragments are given in kilobases

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hybridized with the probe corresponding to the 3'-untranslated exon (Fig. 2). In all these experiments the hybridizing fragment of DRw8 cells coincided with fragments present in DR3, 5, and w6 DNA, but not with fragments in DR4, 7, and w9 DNA (Table 2). These findings suggested that the *DR $\beta$ w8* gene is closely related to one of the genes present in the *DR3*, *DR5*, and *DRw6* haplotypes.

To further investigate this possibility, cosmid clones 4-1, 6-2, and 10-4, containing the *DR $\beta$ 3* genes *I*, *II*, and *III*, respectively (Rollini et al. 1985), were run in parallel

with DR3 and DRw8 genomic DNA on agarose gels after separate digestion with Pst I, Pvu II, and Hind III. The digests were hybridized with the *DR $\beta$*  probes used above, and the results are compiled in Table 2. A representative result obtained after Hind III digestion is shown in Figure 3. It is evident that all hybridizing fragments in the DR3 DNA can be accounted for by fragments in the three cosmids (Table 2 and Fig. 3, lanes 1-3). The DRw8 fragments coincided in all cases with fragments derived from the *DR $\beta$ 3III* gene, i.e., the gene encoding the *DR $\beta$ w52* molecule.

Table 2. Occurrence of bands in DNA from homozygous typing cells of different DR specificities digested with Bam HI, Hind III, Pst I, and Pvu II and hybridized with the *DR $\beta$*  probes from Table 1

Probe and enzyme	Size*	DR specificity										Restriction fragments corresponding to <i>DR<math>\beta</math>3</i> genes*
		1	2	3	4	5	w6	7	w8	w9		
<i>DR<math>\beta</math> 3'UT, Pvu II</i>	3.8		+				ND†					
	4.0				+			+		+		
	4.5		+									
	4.6				+			+		+		
	4.9	+		+		+						$\beta$ I
	5.2			+					+			$\beta$ III
	5.3		+									
	6.8	+										
	11			+		+						$\beta$ II
14				+			+		+			
<i>DR<math>\beta</math> exon 5 and intron, Pst I</i>	0.1				+			+		+		
	0.45	+	+									
	0.52		+	+	+	+	+	+		+		$\beta$ II
	0.57				+			+		+		
	0.65	+										
	0.9		+									
	0.95			+		+	+		+			$\beta$ III
	1.0			+		+	+					$\beta$ I
	1.1				+			+		+		
	2.0				+			+		+		
<i>DR<math>\beta</math> exon 5 and intron, Bam HI</i>	0.3	ND†		+	+	+	+	+		+		ND
	0.6				+			+		+		
	0.85		+	+		+	+		+			
	10				+			+		+		
	14		+	+		+	+					
<i>DR<math>\beta</math> exon 5 and intron, Hind III</i>	0.5			+		+	+					$\beta$ I
	2.2		+									
	2.3			+		+	+					$\beta$ I
	2.4		+		+			+		+		
	2.5			+		+						$\beta$ II
	3.0	+		+		+	+		+			$\beta$ III
	3.2				+			+		+		
	3.6		+									
	6.0	+										
	6.5				+			+		+		
	7.5				+			+		+		

\* Sizes in kilobases

† ND, not done

\* The restriction fragments corresponding to different *DR $\beta$*  genes were determined by Southern blot analysis of representative cosmid clones (see text)

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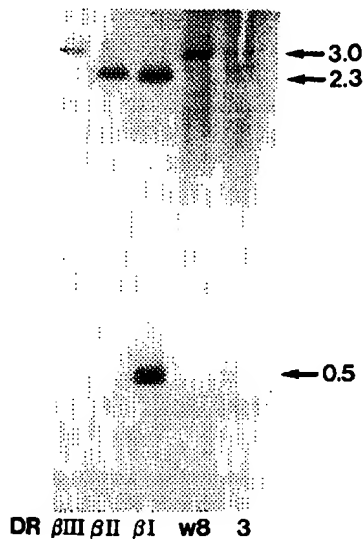


Fig. 3. Comparison of DR3 and DRw8 genomic DNA with cosmid clones DR $\beta$ 3I (4-1), DR $\beta$ 3II (6-2), and DR $\beta$ 3III (10-4), digested with Hind III and hybridized with the DR $\beta$  exon 5 probe

This result clearly indicates that the DR $\beta$ w8 gene is closely related to the DR $\beta$ w52 gene and thus might be a DRw52 allele. In this respect, it is of interest that oligonucleotide probes specific for each of the three DR $\beta$ III alleles identified by DNA sequencing (DRw52a, w52b, and w52c) are negative in hybridization with DRw8 DNA, suggesting the existence of an additional DR $\beta$ III allele (Tiercy et al. 1987). Interestingly, DRw8 cells have been reported to react with some but not all DRw52 sera (Baldwin et al. 1985). This fact and the results above suggest that the DRw8 and DRw52 epitopes are carried by the same DR $\beta$  chain. Serologically, DRw52 and DRw53 are regarded as belonging to the same allelic series (Bach 1985). This notion was recently questioned, since a comparison of the restriction maps and DNA sequences of the DR $\beta$ w53 gene with those of the three DR $\beta$ 3 genes I, II, and III suggested that the DR $\beta$ w53 gene is more closely related to DR $\beta$ 3II than to DR $\beta$ 3III encoding the DR $\beta$ w52 chain (Gorski et al. 1987). It should be noted, however, that careful sequence analyses of the four DR $\beta$ 4 genes showed that extensive exchanges of sequences have occurred among these nonallelic genes, making allelic relationships difficult to establish (Andersson et al. 1987). The sequence of the DR $\beta$ w8 first domain exon was recently reported by Bell and co-workers (1987). A comparison of this sequence with those of other DR $\beta$  first domain exons does not provide any clear answer concerning the relationship of the DR $\beta$ w8 gene. More extensive sequence information is needed to clarify this matter.

The results of the present study suggest that the single DR $\beta$  gene in the DRw8 haplotype is not a DR $\beta$ I gene but

more likely a DR $\beta$ III gene (following the nomenclature for DR $\beta$  genes in the DR3 haplotype). This finding is surprising and has interesting functional implications. Previous studies have indicated that the DR $\beta$ I gene should be the immunologically most important gene in haplotypes which express more than one DR $\beta$  molecule. It has been reported that the major DR $\beta$  polymorphism revealed by serological (DR) or cellular (Dw) methods is encoded by the DR $\beta$ I gene and not by the DR $\beta$ III gene (Gorski et al. 1987). Further, only a minority of all DR-restricted antigen-specific T-cell clones are indeed restricted by allelic products of DR $\beta$ III (C. Irlé, personal communication). Finally, DRw52 and DRw53 molecules have been shown to be expressed at a four- to tenfold lower level than the classical DR molecules (Gregersen et al. 1986, Kratzin et al. 1986, Berdoz et al. 1987). Whether this also is the case for the DRw8/DRw52 molecule is not known. If so, it can be suggested that DR-restricted antigen presentation might be less efficient in DRw8 cells, since they express only a DR $\beta$ III-related gene. Furthermore, it is well established that the repertoire of class II molecules on antigen-presenting cells influences the immune response (Benacerraf and Germain 1978, Zinkernagel and Doherty 1979), and it has been a common view that a broad class II repertoire is beneficial for the individual. If this latter assumption is correct, it would imply that individuals homozygous for DRw8, who only express a single variant of the DR $\beta$  chain, should be at a disadvantage compared with individuals of other known DR genotypes who express two to four different variants of DR $\beta$  chains. To the best of our knowledge there is no data indicating that this should be the case. However, DRw8 homozygotes must be very uncommon because of the fairly low haplotype frequency of DRw8.

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## References

- Albert, E. D., Baur, M. P., and Mayr, W. R. (eds.): *Histocompatibility Testing 1984*, Springer-Verlag, Berlin, Heidelberg, 1985
- Andersson, G., Larhammar, D., Widmark, E., Servenius, B., Peterson, P. A., and Rask, L.: Class II genes of the human major histocompatibility complex. Organization and evolutionary relationship of the DR $\beta$  genes. *J. Biol. Chem.* 262: 8748-8758, 1987
- Bach, F. H.: The HLA class II genes and products: the HLA-D region. *Immunol. Today* 6: 89-94, 1985
- Baldwin, G. C., Mickelson, E. M., Hansen, J. A., Nisperos, B., Antonelli, P., Nepom, B. S., and Nepom, G. T.: Electrophoretic variation between class II molecules expressed on HLA-DRw8 homozygous typing cells reveals multiple distinct haplotypes. *Immunogenetics* 21: 49-60, 1985
- Bell, J. I., Denney, D., Jr., Foster, L., Belt, T., Todd, J. A., and McDewitt, H. O.: Allelic variation in the DR subregion of the hu-

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- Benacerraf, B. and Germain, R. N.: The immune response genes of the major histocompatibility complex. *Immunol. Rev.* 38: 70-119, 1978
- Berdoz, J., Gorski, J., Termijtelen, A.-M., Dayer, J.-M., Irlé, C., Schendel, D., and Mach, B.: Constitutive and induced expression of the individual HLA-DR $\beta$  and  $\alpha$  chain loci in different cell types. *J. Immunol.* 139: 1336-1341, 1987
- Böhme, J., Owerbach, D., Denaro, M., Lernmark, Å., Peterson, P. A., and Rask, L.: Human class II histocompatibility antigen  $\beta$ -chains are derived from at least three loci. *Nature* 301: 82-84, 1983
- Böhme, J., Andersson, M., Andersson, G., Möller, E., Peterson, P. A., and Rask, L.: HLA DR $\beta$  genes vary in number between different DR specificities, whereas the number of DQ $\beta$  genes is constant. *J. Immunol.* 135: 2149-2155, 1985
- Feinberg, A. P. and Vogelstein, B.: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137: 266-267, 1984
- Giles, R. C. and Capra, J. D.: Structure, function and genetics of human class II molecules. *Adv. Immunol.* 27: 1-71, 1985
- Gorski, J., Tosi, R., Strubin, M., Rabourdin-Combe, C., and Mach, B.: Serological and immunochemical analysis of the products of a single HLA DR- $\alpha$  and DR- $\beta$  chain gene expressed in a mouse cell line after DNA-mediated cotransformation reveals that the chain carries a known supertypic specificity. *J. Exp. Med.* 162: 105-116, 1985
- Gorski, J., Rollini, P., and Mach, B.: Structural comparison of the genes of two HLA-DR supertypic groups: the loci encoding DRw52 and DRw53 are not truly allelic. *Immunogenetics* 25: 397-402, 1987
- Gregersen, P. K., Shen, M., Song, Q.-L., Merryman, P., Degar, S., Seki, T., Maccari, J., Goldberg, D., Murphy, H., Schwenzer, J., Wang, C. Y., Winchester, R. J., Nepom, G. T., and Silver, J.: Molecular diversity of HLA-DR4 haplotypes. *Proc. Natl. Acad. Sci. U.S.A.* 83: 2642-2646, 1986
- Grosveld, F. G., Lund, T., Murray, E. J., Mellor, A. L., Dahl, H. H. M., and Flavell, R. A.: The construction of cosmid libraries which can be used to transform eukaryotic cells. *Nucleic Acids Res.* 10: 6715-6732, 1982
- Gustafsson, K., Wiman, K., Emmoth, E., Larhammar, D., Böhme, J., Hyldig-Nielsen, J. J., Ronne, H., Peterson, P. A., and Rask, L.: Mutations and selection in the generation of class II histocompatibility antigen polymorphism. *EMBO J.* 3: 1655-1661, 1984
- Kratzin, H., Götz, H., Thinnies, F. P., Kruse, T., Barnikol, H. U., Wernel, P., and Hilschmann, N.: Structure of human class II antigens expressed by a homozygous lymphoblastoid B cell line. In B. G. Solheim, E. Möller and S. Ferrone (eds.): *HLA Class II Antigens*, pp. 49-70, Springer-Verlag, Berlin, Heidelberg, New York, Tokyo, 1987
- Larhammar, D., Servenius, B., Rask, L., and Peterson, P. A.: Characterization of a HLA-DR $\beta$  pseudogene. *Proc. Natl. Acad. Sci. U.S.A.* 82: 1475-1479, 1985
- Mach, B., Gorski, J., Rollini, P., Berte, C., Amaldi, I., Berdoz, J., and Ucla, C.: Polymorphism and regulation of HLA class II genes of the major histocompatibility complex. *Cold Spring Harbor Symp. Quant. Biol.* 51: 67-74, 1986
- Rigby, P. W. J., Dieckman, M., Rhodes, C., and Berg, P.: Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* 113: 237-251, 1977
- Rollini, P., Mach, B., and Gorski, J.: Linkage map of three HLA-DR $\beta$  chain genes: evidence for a recent duplication event. *Proc. Natl. Acad. Sci. U.S.A.* 82: 7197-7201, 1985
- Tanigaki, N. and Tosi, R.: The genetic control of human Ia alloantigens: a three-loci model derived from the immunochemical analysis of "supertypic" specificities. *Immunol. Rev.* 66: 5-37, 1982
- Tiercy, J.-M., Gorski, J., Jeannet, M., and Mach, B.: Identification and distribution of three serologically undetected alleles of HLA-DR by oligonucleotide typing. *Proc. Natl. Acad. Sci. U.S.A.*, in press, 1987
- Zinkernagel, R. M. and Doherty, P. C.: MHC-restricted T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function and responsiveness. *Adv. Immunol.* 27: 221-292, 1979

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